Lucas 09/901,572

=> d his 1 STIC Search

(FILE 'MEDLINE, HCAPLUS, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 13:13:03 ON 25 AUG 2003)
L6 15 S L4 OR L5

=> d que 16
L1 112 SEA (ELIMINAT? OR MODIF? OR PREVENT? OR AVOID? OR PROHIBIT?)(5A)(N(A) GLYCOSIDAT? OR GLYCOSIDAT?)
L2 23 SEA NON(A)(GLYCOSIDAT? OR N(A) GLYCOSIDAT?)
L3 133 SEA L1 OR L2
L4 8 SEA L3 AND ASPARAGINE#
L5 8 SEA L3 AND (PROKARYOT? OR PROCARYOT? OR MYCOPLASM? OR BACTERI?)

L6 15 SEA L4 OR L5

=> d ibib abs 16 1-15

L6 ANSWER 1 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:241881 HCAPLUS
DOCUMENT NUMBER: 138:249779
TITLE: Selective **modification** of coding sequences to **eliminate glycosidation** sites of gene products for vaccines
INVENTOR(S): Okuda, Takashi; Saito, Shuji; Dorsey, Kristi M.; Tsuzaki, Yoshinari
PATENT ASSIGNEE(S): Japan
SOURCE: U.S. Pat. Appl. Publ., 53 pp., Cont.-in-part of U.S. Ser. No. 901,572.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003059799	A1	20030327	US 2002-131591	20020425
JP 2003088391	A2	20030325	JP 2002-195083	20020703
EP 1275716	A2	20030115	EP 2002-254879	20020711
EP 1275716	A3	20030305		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

PRIORITY APPLN. INFO.: US 2001-901572 A2 20010711
US 2002-131591 A 20020425

AB A method of prepg. glycosidation-free variants of a protein in a microbial host is described. The glycosidation-free proteins are for use in vaccines, e.g. using a viral expression vectors in vector vaccines. N-linked glycosidation sites NXB (N = **asparagine**, X = any amino acid except proline; B = serine or threonine) are modified so that they are no longer recognized for glycosidation. The genes for the TTM-1 and M11 glycoproteins of **Mycoplasma** gallisepticum were **modified** to remove **N-glycosidation** sites and introduced into fowlpox and gallid herpesvirus vectors. The vectors directed synthesis of the non-glycosylated form of the protein in chick embryo fibroblast cultures. Five week-old chicks were inoculated with the fowlpox vector carrying the TTM-1 gene 104 pfu. Two weeks later, they were challenged with M. gallisepticum 4.8.times.104 cfu. Control chickens

Search completed by David Schreiber 308-4292

showed an av. of 2.53 tracheal lesions each. Chickens inoculated with the vector carrying the wild-type TTM-1 gene showed 2.78 tracheal lesions. Those vaccinated with the gene for the **non-glycosidated** form showed 1.96 tracheal lesions.

L6 ANSWER 2 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:594820 HCAPLUS
DOCUMENT NUMBER: 127:244012
TITLE: A method of screening multiple libraries for genes or cDNAs encoding proteins that interact to form homo- or hetero-oligomers
INVENTOR(S): Ilag, Vic; Ge, Liming
PATENT ASSIGNEE(S): Morphosys Gesellschaft Fur Proteinoptimierung m.b.H., Germany; Ilag, Vic; Ge, Liming
SOURCE: PCT Int. Appl., 104 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9732017	A1	19970904	WO 1997-EP931	19970226
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 883686	A1	19981216	EP 1997-905095	19970226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000505306	T2	20000509	JP 1997-530595	19970226

PRIORITY APPLN. INFO.: EP 1996-102852 19960226
WO 1997-EP931 19970226

AB A method of identifying genes or cDNAs encoding proteins that interact with one another using two distinct expression libraries is described. The method uses two libraries in different expression vectors and the interaction is detected by the appearance of a distinct phenotype. If one of the vectors is a **bacteriophage** or virus, the preferred phenotype affected is infectivity. One method of making a non-infectious **bacteriophage** infective is to use the binding to reconstitute a protein necessary for infectivity, e.g. by using a fusion protein with the N-terminal domain of the gene III protein of filamentous **bacteriophages**. The method may also be used to investigate interactions based upon post-translational **modifications** such as phosphorylation, **glycosidation**, or methylation (no data). Preferred hosts are Escherichia coli or Neisseria gonorrheae that present a component of the complex on the cell surface as a fusion protein with a flagellar protein, the lamB protein, peptidoglycan-assocd. lipoprotein, or the OmpA gene product. One of the fusion products may be further labeled with an affinity tag to simplify purifn. and it can also be used in ordered array gene banks.

L6 ANSWER 3 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:878345 HCAPLUS
DOCUMENT NUMBER: 123:279163
TITLE: Posttranslational modifications of bovine osteopontin: identification of twenty-eight phosphorylation and three O-glycosylation sites
AUTHOR(S): Soerensen, Esben S.; Hoejrup, Peter; Petersen, Torben E.

CORPORATE SOURCE: Protein Chemistry Lab., Univ. of Aarhus, Science Park, DK-8000, Den.
SOURCE: Protein Science (1995), 4(10), 2040-9
CODEN: PRCIEI; ISSN: 0961-8368
PUBLISHER: Cambridge University Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Osteopontin (OPN) is a multiphosphorylated glycoprotein found in bone and other normal and malignant tissues, as well as in the physiol. fluids urine and milk. The present study demonstrates that bovine milk osteopontin is phosphorylated at 27 serine residues and 1 threonine residue. Phosphoamino acids were identified by a combination of amino acid anal., sequence anal. of S-ethylcysteine-derivatized phosphopeptides, and mass spectrometric anal. Twenty-five phosphoserines and one phosphothreonine were located in Ser/Thr-X-Glu/Ser(P)/Asp motifs, and two phosphoserines were found in the sequence Ser-X-X-Glu/Ser(P). These sequences motifs are identical with the recognition sequences of mammary gland casein kinase and casein kinase II, resp. Examn. of the phosphorylation pattern revealed that the phosphorylations were clustered in groups of approx. three spanned by unphosphorylated regions of 11-32 amino acids. This pattern is probably of importance in the multiple functions of OPN involving interaction with Ca2+ and inorg. calcium salts. Furthermore, three O-glycosylated threonines (Thr 115, Thr 124, and Thr 129) have been identified in a threonine- and proline-rich region of the protein. Three putative N-glycosylation sites (Asn 63, Asn 85, and Asn 193) are present in bovine osteopontin, but sequence and mass spectrometric anal. showed that none of these **asparagines** were glycosylated in bovine mammary gland osteopontin. Alignment anal. showed that the majority of the phosphorylation sites in bovine osteopontin as well as all three O-glycosylation sites were conserved in other mammalian sequences. This conservation of serines, even in otherwise less well-conserved regions of the protein, indicates that the phosphorylation of osteopontin at specific sites is essential for the function of the protein.

L6 ANSWER 4 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1995:748112 HCAPLUS
DOCUMENT NUMBER: 123:165502

TITLE: Elimination of glycosylation heterogeneity affecting heparin affinity of recombinant human antithrombin III by expression of a .beta.-like variant in baculovirus-infected insect cells

AUTHOR(S): Ersdal-Badju, Eva; Lu, Aiqin; Peng, Xiaoming; Picard, Veronique; Zendehrouh, Pedram; Turk, Boris; Bjoerk, Ingemar; Olson, Steven T.; Bock, Susan C.
CORPORATE SOURCE: Sol Sherry Thrombosis Res. Cent., Temple Univ., Philadelphia, PA, 19140, USA

SOURCE: Biochemical Journal (1995), 310(1), 323-30
CODEN: BIJOAK; ISSN: 0264-6021
PUBLISHER: Portland Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB To promote homogeneity of recombinant antithrombin III interactions with heparin, an **asparagine**-135 to alanine substitution mutant was expressed in baculovirus-infected insect cells. The N135A variant does not bear an N-linked oligosaccharide on residue 135 and is therefore similar to the .beta. isoform of plasma antithrombin. Purified bv.hat3.N135A is homogeneous with respect to mol. mass, charge and elution from immobilized heparin. Second-order rate consts. for thrombin and

factor Xa inhibition detd. in the absence and presence of heparin are in good agreement with values established for plasma antithrombin and these enzymes. Based on far- and near-UV CD, bv.hat3.N135A has a high degree of conformational similarity to plasma antithrombin. Near-UV CD, absorption difference and fluorescence spectroscopy studies indicate that it also undergoes an identical or very similar conformational change upon heparin binding. The Kds of bv.hat3.N135A for high-affinity heparin and pentasaccharide were detd. and are in good agreement with those of the plasma .beta.-antithrombin isoform. The demonstrated similarity of bv.hat3.N135A and plasma antithrombin interactions with target proteinases and heparins suggest that it will be a useful base mol. for investigating the structural basis of antithrombin III heparin cofactor activity.

L6 ANSWER 5 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:500320 HCAPLUS
DOCUMENT NUMBER: 122:262235
TITLE: Post-translational and activation-dependent modifications of the G protein-coupled thrombin receptor
AUTHOR(S): Vouret-Craviari, Valerie; Grall, Dominique; Chambard, Jean Claude; Rasmussen, Ulla B.; Pouyssegur, Jacques; Van Obberghen-Schilling, Ellen
CORPORATE SOURCE: Cent. Biochim., CNRS, Nice, 06108, Fr.
SOURCE: Journal of Biological Chemistry (1995), 270(14), 8367-72
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The purpose of the present study was to analyze the post-translational and activation-dependent modifications of the G protein-coupled thrombin receptor. A human receptor cDNA was engineered to encode an epitope tag derived from the vesicular stomatitis virus glycoprotein at the COOH terminus of the receptor and expressed in human embryonic kidney 293 cells. We show here that the mature receptor is a glycosylated protein with an apparent mol. mass ranging from 68 to 80 kDa by SDS-polyacrylamide gel electrophoresis. Removal of **asparagine**-linked oligosaccharides with N-glycosidase F leads to the appearance of a 36-40-kDa receptor species. The current model for receptor activation by thrombin involves specific hydrolysis of the arginine-41/serine-42 (Arg-41/Ser-42) peptide bond. Cleavage of the receptor by thrombin was demonstrated directly by Western analyses performed on membranes and glycoprotein-enriched lysates from transfected cells. Whereas thrombin treatment of cells results in increased mobility of the receptor in SDS-polyacrylamide gel electrophoresis, we found that their treatment with the thrombin receptor agonist peptide leads to a decrease in thrombin receptor mobility due, in part, to phosphorylation. The serine proteases trypsin and plasmin also cleave and activate the receptor similar to thrombin, whereas chymotrypsin cleaves the receptor at a site distal to Arg-41, thus rendering it unresponsive to thrombin while still responsive to thrombin receptor agonist peptide.

L6 ANSWER 6 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:486446 HCAPLUS
DOCUMENT NUMBER: 122:262188
TITLE: Lectins and also **bacteria** modify the glycosylation of gut surface receptors in the rat
AUTHOR(S): Pusztai, Arpad; Ewen, Stanley W. B.; Grant, George;

Peumans, Willy J.; Van Damme, Els J. M.; Coates, Marie E.; Bradocz, Susan
CORPORATE SOURCE: Rowett Res. Inst., Bucksburn, Aberdeen, AB1 2ZX, UK
SOURCE: Glycoconjugate Journal (1995), 12(1), 22-35
CODEN: GLJOEW; ISSN: 0282-0080
PUBLISHER: Chapman & Hall
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Oral exposure to lectins or the presence or absence of **bacteria** in the rat small intestine were shown by histol. methods using anti-lectin antibodies or digoxigenin-labeled lectins to have major effects on the state of glycosylation of lumenal membranes and cytoplasmic glycoconjugates of epithelial cells. Taken together with the dramatic effects of exposure to lectins or gut function, metab. and **bacterial** ecol., this can be used as a basis for new perspectives of biomedical manipulations to improve health.

L6 ANSWER 7 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1995:377238 HCAPLUS
DOCUMENT NUMBER: 122:182005
TITLE: Cloned DNA encoding a UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase and acceptor peptides for the enzyme
INVENTOR(S): Elhammer, Ake P.; Homa, Fred L.
PATENT ASSIGNEE(S): Upjohn Co., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9426906	A2	19941124	WO 1994-US2552	19940317
WO 9426906	A3	19960613		
W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, TJ, TT, UA, US, UZ, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9466632	A1	19941212	AU 1994-66632	19940317
EP 698103	A1	19960228	EP 1994-915336	19940317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
EP 726318	A1	19960814	EP 1996-104017	19940317
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 09501044	T2	19970204	JP 1994-525397	19940317
US 5910570	A	19990608	US 1997-967508	19971111

PRIORITY APPLN. INFO.:
US 1993-63186 19930514
EP 1994-915336 19940317
WO 1994-US2552 19940317
US 1995-602830 19951113

AB The present invention relates to a method for the isolation and expression of a glycosyltransferase enzyme for use in the synthesis of oligosaccharide or polysaccharide structures on glycoproteins, glycolipids, or as free mols. The gene coding for the enzyme N-acetylgalctosaminyltransferase and the polypeptide sequence of the acceptor peptide for the N-acetylgalactosaminyltransferase were isolated and used for the control of protein glycosylation. Thus, the title enzyme

was isolated from bovine colostrum; its cDNA was isolated and characterized by std. techniques. A secreted, sol. form of the enzyme was engineered in which the sequences coding for the cytoplasmic and membrane-spanning domains of the full-length cDNA (141 nucleotides) were replaced with sequences that code for the honeybee melittin signal peptide and five linker amino acids (78 nucleotides). Wild-type and sol. enzymes were cloned and expressed in Sf9 cells. Acceptor peptides included PPASTSAPG and PPASSSAPG were glycosylated by the enzyme with Vmax/Km values of 301 and 8.5 M-1s-1.

L6 ANSWER 8 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1994:263048 HCAPLUS
DOCUMENT NUMBER: 120:263048
TITLE: Plasmids modified with advanced glycosylation end products and the capture of transposon-modified DNA with them.
INVENTOR(S): Bucala, Richard J.; Lee, Annette T.; Cerami, Anthony
PATENT ASSIGNEE(S): The Rockefeller University, USA
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9402599	A1	19940203	WO 1993-US6754	19930719
W: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9346833	A1	19940214	AU 1993-46833	19930719
PRIORITY APPLN. INFO.:			US 1992-920985	A2 19920722
			WO 1993-US6754	W 19930719

AB Nucleic acids modified by reaction with sugars to form advanced glycosylation end products (AGE) or a compd. that forms advanced glycosylation end products are described for use in transposon capture. These AGE-modified nucleic acids are typically plasmids that can be introduced into cells, where they may capture transposons present in the cell. Deletion in the AGE-plasmids is used as an indicator of transposon activity. The AGE-modification of the plasmids may activate the transposons which are captured. The invention also encompasses a no. of assays wherein the transfected cells are evaluated for transposon/deletion activity. A polyoma-based shuttle vector pPy35 carrying a lacI mutagenesis marker was **modified** by advanced **glycosidation** in vitro and introduced into X63Ag8.653 cells. Episomal DNA was recovered after selection and assayed for lacI mutation by .alpha.-complementation. An Alu-contg. sequence of 853 bp occurred at a 60-fold greater frequency in the glycosidated plasmids than in controls with the mutagenesis rate of 0.1% for control plasmids rising to 28% for the AGE plasmids. The use of the method to study the effects of maternal diabetic hyperglycemia on embryonic DNA damage was demonstrated.

L6 ANSWER 9 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1991:201114 HCAPLUS
DOCUMENT NUMBER: 114:201114
TITLE: Recombinant manufacture of a tissue plasminogen

activator analog with a prolonged serum clearance time

INVENTOR(S): Stern, Anne; Kohnert, Ulrich; Rudolph, Rainer; Fischer, Stephan; Martin, Ulrich

PATENT ASSIGNEE(S): Boehringer Mannheim G.m.b.H., Germany

SOURCE: Ger. Offen., 16 pp.
CODEN: GWXXBX

DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 3903581	A1	19900816	DE 1989-3903581	19890207
DD 291779	A5	19910711	DD 1990-337557	19900202
IL 93280	A1	19950831	IL 1990-93280	19900205
CA 2025900	AA	19900808	CA 1990-2025900	19900206
CA 2025900	C	19990119		
EP 382174	A1	19900816	EP 1990-102329	19900206
EP 382174	B1	19950809		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL				
WO 9009437	A1	19900823	WO 1990-EP194	19900206
W: AU, CA, FI, HU, JP, KR, NO, SU, US				
AU 9050470	A1	19900905	AU 1990-50470	19900206
AU 623228	B2	19920507		
ZA 9000861	A	19901128	ZA 1990-861	19900206
JP 03500724	T2	19910221	JP 1990-502957	19900206
JP 2559538	B2	19961204		
HU 58813	A2	19920330	HU 1990-1644	19900206
HU 218092	B	20000528		
ES 2031804	T3	19951101	ES 1990-102329	19900206
CZ 281836	B6	19970212	CZ 1990-558	19900206
RU 2107094	C1	19980320	RU 1990-4831496	19900206
SK 279029	B6	19980506	SK 1990-558	19900206
NO 9004211	A	19900927	NO 1990-4211	19900927
US 5223256	A	19930629	US 1990-585129	19900928
KR 9708485	B1	19970524	KR 1990-72203	19900929
LV 10302	B	19950420	LV 1993-448	19930601
US 5676947	A	19971014	US 1994-217617	19940325
US 5854048	A	19981229	US 1996-600396	19960212

PRIORITY APPLN. INFO.:

DE 1989-3903581	A	19890207
WO 1990-EP194	A	19900206
US 1990-527498	B1	19900523
US 1990-585129	A1	19900928
US 1992-892629	B1	19920602
US 1992-968171	B3	19921029
US 1993-130005	B2	19930930
US 1993-165577	B1	19931213
US 1994-217617	A3	19940325

AB A tissue plasminogen activator analog contg. the second kringle domain and the protease domain, that is not glycosidated in vivo and that has a longer serum clearance time than the wild-type enzyme is manufd. by expression of the gene in Escherichia coli. The gene was expressed from the tac promoter in a low copy-no. plasmid (based upon pACYC177) with the protein accumulated as inclusion bodies. The protein was recovered by std. methods (guanidinium chloride solubilization, affinity chromatog. using Erythrina trypsin inhibitor). The protein did not bind fibrin significantly. The serum half-life of this analog in rabbits was 10.3 min. vs. 2.1 for a com. prepn. (Actilyse).

L6 ANSWER 10 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:494401 HCAPLUS
DOCUMENT NUMBER: 113:94401
TITLE: **Modification** of the **glycosidation** of proteins in vitro to enhance stability in the bloodstream
INVENTOR(S): Bergh, Michel L. E.; Hubbard, S. Catherine; Rasmussen, James R.
PATENT ASSIGNEE(S): Massachusetts Institute of Technology, USA
SOURCE: U.S., 22 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4925796	A	19900515	US 1986-837604	19860307
US 5272066	A	19931221	US 1991-785913	19911104
PRIORITY APPLN. INFO.:			US 1986-837604	19860307
			US 1989-337294	19890313

AB Glycosidation patterns that improve the serum stability of exogenous proteins administered as therapeutics are introduced into the protein after enzymic or chem. deglycosidation or after biosynthesis of the protein in the presence of glycosidation inhibitors to leave asparaginyl N-monosaccharides. Glycosidation is then effected using appropriate glycosyl transferases. SDS-denatured yeast external invertase 250 was deglycosylated by digestion with endoglycosidase H 0.3 .mu.g (20h, 27.degree.) and jack bean .alpha.-mannosidase (20 milliunits, 17 h, 37.degree.). The deglycosylated protein was glycosidated using UDP-[3H] galactoside as substrated for bovine milk UDP:GlcNac .beta.1.fwdarw.4 galactosyltransferase. The precursor was incorporated into the protein with a concomitant increase in mol. wt. (SDS-PAGE). The galactosidated protein was then similarly sialylated using CMP-[14C]NeuAc as substrate for bovine colostrum sialyl transferase. Bovine serum albumin derivs. with different glycosidation patterns showed different levels of uptake by mouse peritoneal macrophages.

L6 ANSWER 11 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1990:476660 HCAPLUS
DOCUMENT NUMBER: 113:76660
TITLE: Recovery of **non-glycosidated**, reduced human interleukin 2 from **bacterial** inclusion granules
INVENTOR(S): Lando, Danielle; Riberon, Philippe; Abecassis, Pierre Yves
PATENT ASSIGNEE(S): Roussel-UCLAF, Fr.
SOURCE: Eur. Pat. Appl., 18 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 353150	A1	19900131	EP 1989-402124	19890726

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EP 353150	B1	19940831		
R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
FR 2635527	A1	19900223	FR 1988-10184	19880728
FR 2635527	B1	19920612		
IL 90975	A1	19941128	IL 1989-90975	19890714
ZA 8905419	A	19900926	ZA 1989-5419	19890717
ES 2058573	T3	19941101	ES 1989-402124	19890726
DK 8903704	A	19900129	DK 1989-3704	19890727
FI 8903587	A	19900129	FI 1989-3587	19890727
FI 96209	B	19960215		
FI 96209	C	19960527		
HU 51647	A2	19900528	HU 1989-3827	19890727
HU 207099	B	19930301		
JP 02209896	A2	19900821	JP 1989-192769	19890727
JP 3016793	B2	20000306		
AU 8939024	A1	19900201	AU 1989-39024	19890728
AU 624625	B2	19920618		
CN 1042377	A	19900523	CN 1989-106242	19890728
CN 1036532	B	19971126		
RU 2105011	C1	19980220	RU 1992-5010517	19920108
US 5874076	A	19990223	US 1995-544092	19951017
US 5814314	A	19980929	US 1996 601434	19960212
PRIORITY APPLN. INFO.:			FR 1988-10184 A	19880728
			US 1989-384986 B1	19890724
			US 1992-869803 B1	19920416
			US 1994-204650 B3	19940301

AB Recombinant, biol. active interleukin-2 (IL-2) suitable for use in pharmaceuticals is prepd. from Escherichia coli inclusion bodies by solubilization of an inclusion body prepn. in a buffer contg. guanidinium hydrochloride and a thiol reagent. The IL-2 is then pptd. by diln. of the ext., resolubilized with a buffer soln. contg. acetonitrile (20%) and trifluoroacetic acid (0.1%). The IL-2 is then recovered from this soln. by rounds of HPLC using different solvent gradients for elution in each step. Yields from a 10-L fermn. broth recombinant E. coli were 150-300 mg IL-2 with a biol. activity of 1.3 .times. 107 units/mg. Formulations for use in injection (100 .mu.g/mL IL-2, 50 mg/mL mannitol, and in perfusion were described.

L6 ANSWER 12 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER:	1988:145754 HCAPLUS
DOCUMENT NUMBER:	108:145754
TITLE:	Polylactosaminoglycan modification of a small integral membrane glycoprotein, influenza B virus NB
AUTHOR(S):	Williams, Mark A.; Lamb, Robert A.
CORPORATE SOURCE:	Dep. Biochem., Mol. Biol. Cell Biol., Northwestern Univ., Evanston, IL, 60208, USA
SOURCE:	Molecular and Cellular Biology (1988), 8(3), 1186-96 CODEN: MCEBD4; ISSN: 0270-7306
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB The structure of the carbohydrate components of NB of influenza B virus was investigated. The carbohydrate chains of NB are processed from the high-mannose form (NB18) to a heterogeneous form of much higher mol. wt., designated NBp. Selection of this carbohydrate-contg. form of NB with Datura stramonium lectin, its susceptibility to digestion by endo-.beta.-galactosidase, and detn. of the size of NBp glycopeptides by gel filtration chromatog. suggested that the increase in mol. wt. is due to processing to polylactosaminoglycan. Investigation of the polypeptides produced by influenza B/Lee/40 virus infection of several cell types and

another strain of influenza B virus suggested that the signal for modification to polylactosaminoglycan is contained in NB. Expression of mutants of NB lacking either 1 or both of the normal N-terminal sites of **asparagine**-linked glycosylation indicated that both carbohydrate chains are modified to contain polylactosaminoglycan. NBp and a small amt. of unprocessed NB18 are expressed at the infected-cell surface, as detd. by digestion of the surfaces of intact cells with various endoglycosidases. Unglycosylated NB, expressed either in influenza B virus-infected cells treated with tunicamycin or in cells expressing the NB mutant lacking both N-linked glycosylation sites, was expressed at the cell surface, indicating that NB does not require carbohydrate addn. for transport.

L6 ANSWER 13 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1987:513117 HCAPLUS
DOCUMENT NUMBER: 107:113117
TITLE: Identification of the post-translational modifications of the core-specific lectin. The core-specific lectin contains hydroxyproline, hydroxylysine, and glucosylgalactosylhydroxylysine residues
AUTHOR(S): Colley, Karen J.; Baenziger, Jacques U.
CORPORATE SOURCE: Sch. Med., Washington Univ., St. Louis, MO, 63110, USA
SOURCE: Journal of Biological Chemistry (1987), 262(21), 10290-5
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The core-specific lectin (CSL) synthesized and secreted by rat hepatocytes and the rat hepatoma H-4-II-E shows affinity for mannose and N-acetylglucosamine residues in the core region of **asparagine**-linked oligosaccharides. The CSL undergoes 2 stages of posttranslational modification which result in an increase in its mol. wt. (Mr) from 24,000 to 26,000 detd. by SDS-PAGE. The lectin undergoes hydroxylation of proline and lysine, and the hydroxylysine is glycosylated to form glucosylgalactosylhydroxylysine (GlcGalHyLys). CSL metabolically labeled with [3H]lysine and [3H]proline contains hydroxylated forms of proline and lysine. The mature form of the lectin can also be metabolically labeled with [3H]galactose. .alpha.,.alpha.'-Dipyridyl, an inhibitor of collagen prolyl and lysyl hydroxylases, prevents the metabolic incorporation of [3H]galactose and the posttranslational increases in the Mr of the CSL, indicating that both events are dependent upon hydroxylation of proline and lysine. Virtually all of the hydroxylysine present in the CSL is recovered as glucosylgalactosylhydroxylysine after alk. hydrolysis. The posttranslational modifications of the CSL place it in a select family of secreted proteins which contain collagenlike sequences, including the pulmonary surfactant proteins, complement component C1q, and 18 S asym. form of acetylcholinesterase.

L6 ANSWER 14 OF 15 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1985:521307 HCAPLUS
DOCUMENT NUMBER: 103:121307
TITLE: SV40 T antigen and the exocytotic pathway
AUTHOR(S): Sharma, Sri; Rodgers, Linda; Brandsma, Janet; Gething, Mary Jane; Sambrook, Joe
CORPORATE SOURCE: Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724, USA
SOURCE: EMBO Journal (1985), 4(6), 1479-89
CODEN: EMJODG; ISSN: 0261-4189
DOCUMENT TYPE: Journal

Search completed by David Schreiber 308-4292

LANGUAGE: English

AB A chimeric gene consisting of DNA coding for the 15-amino acid signal peptide of influenza virus hemagglutinin and the C-terminal 694 amino acids of SV40 large T antigen was inserted into a bovine papilloma virus (BPV) expression vector and introduced into NIH-3T3 cells. Cell lines were obtained that express high levels (.apprx.5 .times. 106 mols./cell) of the chimeric protein (HA-T antigen). The biochem. properties and intracellular localization of HA-T antigens were compared with those of wild-type T antigen. Wild-type T antigen is located chiefly in the cell nucleus, although a small fraction is detected on the cell surface. By contrast, HA-T antigen is found exclusively in the endoplasmic reticulum (ER). During biosynthesis, HA-T antigen is co-translationally translocated across the membrane of the ER, the signal peptide is cleaved and a mannose-rich oligosaccharide is attached to the polypeptide (T antigen contains 1 potential N-linked glycosylation site at **asparagine**-154). HA-T antigen does not become terminally glycosylated or acylated and little or none reaches the cell surface. Apparently, T antigen is incapable of being transported along the exocytotic pathway. To explain the presence of wild-type T antigen on the surface of SV40-transformed cells, an alternative route is proposed involving transport of T antigen from the nucleus to the cell surface.

L6 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:490815 BIOSIS
DOCUMENT NUMBER: PREV199800490815
TITLE: Synthesis of aryl 3-O-beta-cellobiosyl-beta-D-glucopyranosides for reactivity studies of 1,3-1,4-beta-glucanases.
AUTHOR(S): Planas, Antoni (1); Abel, Mireia; Millet, Oscar; Palasi, Josep; Pallares, Cristina; Viladot, Josep-Lluis
CORPORATE SOURCE: (1) Lab. Biochem., Dep. Organic Chem., Inst. Quimica de Sarria, Univ. Ramon Lull, Via Augusta 390, 08017-Barcelona Spain
SOURCE: Carbohydrate Research, (Aug., 1998) Vol. 310, No. 1-2, pp. 53-64.
ISSN: 0008-6215.
DOCUMENT TYPE: Article
LANGUAGE: English

AB A series of substituted aryl beta-glycosides derived from 3-O-beta-cellobiosyl-D-glucopyranose with different phenol-leaving group abilities as measured by the pKa of the free phenol group upon enzymatic hydrolysis has been synthesized. Aryl beta-glycosides with a pKa of the free phenol leaving group> 5 were prepared by phase-transfer glycosidation of the per-O-acetylated alpha-glycosyl bromide with the corresponding phenol, whereas the 2,4-dinitrophenyl beta-glycoside was obtained by condensation of 1-fluoro-2,4-dinitrobenzene with the partially acetylated trisaccharide followed by acid de-O-acetylation. The aryl beta-glycosides have been used for reactivity studies of the wild-type Bacillus licheniformis 1,3-1,4-beta-D-glucan 4-glucanohydrolase. The Hammett plot log kcat versus pKa is biphasic with an upward curvature at low pKa values suggesting a change in transition-state structure depending on the aglycon.

Search completed by David Schreiber 308-4292

Lucas 09/901,572

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=> d his 1

     (FILE 'MEDLINE, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT 10:26:16
     ON 25 AUG 2003)

     FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, SCISEARCH, AGRICOLA' ENTERED AT
     10:40:38 ON 25 AUG 2003
L31          23 DUP REM L11 L20 L21 L22 L27 L29 L30 (31 DUPLICATES REMOVED)

=> d que 131
L1         2197 SEA FILE=HCAPLUS OKUDA T?/AU
L2         7471 SEA FILE=HCAPLUS SAITO S?/AU
L3         1432 SEA FILE=HCAPLUS MOORE K?/AU
L4           52 SEA FILE=HCAPLUS TSUZAKI Y?/AU
L5        11136 SEA FILE=HCAPLUS (L1 OR L2 OR L3 OR L4)
L6           45 SEA FILE=HCAPLUS L5 AND (N(A)GLYCOSYL? OR GLYCOSYL?)
L7            4 SEA FILE=HCAPLUS L6 AND ASPARAGINE
L8              TRANSFER  L7 1-4 RN :     164 TERMS
L9          164 SEA FILE=REGISTRY L8
L10           6 SEA FILE=REGISTRY L9 AND N[-P][ST]/SQSP
L11           2 SEA FILE=HCAPLUS L10
L12        1961 SEA FILE=HCAPLUS (ELIMINAT? OR MODIF? OR PREVENT? OR AVOID? OR
                PROHIBIT?)(5A)(N(A)GLYCOSYL? OR GLYCOSYL?)
L13         755 SEA FILE=HCAPLUS (NON(A)(GLYCOSYL? OR N(A)GLYCOSYL?))
L14        2690 SEA FILE=HCAPLUS L12 OR L13
L15         184 SEA FILE=HCAPLUS L14 AND ASPARAGINE#
L16             TRANSFER  L15 1-184 RN :     988 TERMS
L17         988 SEA FILE=REGISTRY L16
L18         115 SEA FILE=REGISTRY L17 AND N[-P][ST]/SQSP
L19          63 SEA FILE=HCAPLUS L18
L20           2 SEA FILE=HCAPLUS L19 AND (PROCARYOT? OR PROKARYOT? OR MYCOPLASM
                ?)
L21           2 SEA FILE=HCAPLUS L15 AND (PROCARYOT? OR PROKARYOT? OR MYCOPLASM
                ?)
L22           6 SEA FILE=HCAPLUS L15 AND BACTERI?
L23        5207 SEA (ELIMINAT? OR MODIF? OR PREVENT? OR AVOID? OR PROHIBIT?)(5A
                )(N(A) GLYCOSYL? OR GLYCOSYL?)
L24        3057 SEA (NON(A)(GLYCOSYL? OR N(A) GLYCOSYL?))
L25        8179 SEA L23 OR L24
L26         564 SEA L25 AND ASPARAGINE#
L27          36 SEA L26 AND (PROCARYOT? OR PROKARYOT? OR MYCOPLASM? OR
                BACTERI?)
L29           5 SEA L26 AND (PROCARYOT? OR PROKARYOT? OR MYCOPLASM? OR
                BACTERI?)(5A)(PROTEIN# OR POLYPEPTIDE# OR PEPTIDE# OR ANTIGEN?)

L30           1 SEA L26 AND (PROCARYOT? OR PROKARYOT? OR MYCOPLASM? OR
                BACTERI?)(5A)(DNA OR RNA OR RIBONUCLEIC OR DEOXYRIBONUCLEIC OR
                NUCLEIC OR RECOMBINAN?)
L31          23 DUP REM L11 L20 L21 L22 L27 L29 L30 (31 DUPLICATES REMOVED)


=> d ibib abs 131 1-23

L31  ANSWER 1 OF 23  HCAPLUS  COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER:         2003:241881  HCAPLUS
DOCUMENT NUMBER:          138:249779
TITLE:                    Selective modification of coding sequences to
                          eliminate glycosidation sites of gene products for
                          vaccines
```

Search completed by David Schreiber 308-4292

INVENTOR(S): Okuda, Takashi; Saito, Shuji; Dorsey, Kristi M.; Tsuzaki, Yoshinari
PATENT ASSIGNEE(S): Japan
SOURCE: U.S. Pat. Appl. Publ., 53 pp., Cont.-in-part of U.S. Ser. No. 901,572.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003059799	A1	20030327	US 2002-131591	20020425
JP 2003088391	A2	20030325	JP 2002-195083	20020703
EP 1275716	A2	20030115	EP 2002-254879	20020711
EP 1275716	A3	20030305		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

PRIORITY APPLN. INFO.: US 2001-901572 A2 20010711
US 2002-131591 A 20020425

AB A method of prepg. glycosidation-free variants of a protein in a microbial host is described. The glycosidation-free proteins are for use in vaccines, e.g. using a viral expression vectors in vector vaccines. N-linked glycosidation sites NXB (N = asparagine, X = any amino acid except proline; B = serine or threonine) are modified so that they are no longer recognized for glycosidation. The genes for the TTM-1 and M11 glycoproteins of Mycoplasma gallisepticum were modified to remove N-glycosidation sites and introduced into fowlpox and gallid herpesvirus vectors. The vectors directed synthesis of the non-glycosylated form of the protein in chick embryo fibroblast cultures. Five week-old chicks were inoculated with the fowlpox vector carrying the TTM-1 gene 104 pfu. Two weeks later, they were challenged with M. gallisepticum 4.8.times.104 cfu. Control chickens showed an av. of 2.53 tracheal lesions each. Chickens inoculated with the vector carrying the wild-type TTM-1 gene showed 2.78 tracheal lesions. Those vaccinated with the gene for the non-glycosidated form showed 1.96 tracheal lesions.

L31 ANSWER 2 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2
ACCESSION NUMBER: 2003:40197 HCAPLUS
DOCUMENT NUMBER: 138:84445
TITLE: Modification of prokaryotic DNA molecule at the N-glycosylation site, produces a non-N-glycosylated antigen protein and its use via recombinant virus as vaccines
INVENTOR(S): Okuda, Takashi; Saito, Shuji; Dorsey, Kristi M.; Tsuzaki, Yoshinari
PATENT ASSIGNEE(S): Zeon Corporation, Japan
SOURCE: Eur. Pat. Appl., 70 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1275716	A2	20030115	EP 2002-254879	20020711
EP 1275716	A3	20030305		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK

US 2003059799 A1 20030327 US 2002-131591 20020425

PRIORITY APPLN. INFO.: US 2001-901572 A 20010711

US 2002-131591 A 20020425

AB There is provided a DNA mol. derived from a prokaryotic cell in which at least one of the DNA regions encoding NXB (N is asparagine, X is any amino acid other than proline, and B is serine or threonine) has been modified so that no N-glycosylation occurs during the expression in a eukaryotic cell. The modified DNA mol. at the N-glycosylation site, produces a non-N-glycosylated protein, which thereby exhibits a high immunogenicity when, for example, it is allowed to produce, in a eukaryotic cell, an antigen protein derived from a prokaryotic cell.

L31 ANSWER 3 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3

ACCESSION NUMBER: 2003:403442 HCAPLUS

TITLE: A novel vascular endothelial growth factor-directed therapy that selectively activates cytotoxic prodrugs

AUTHOR(S): Spooner, R. A.; Friedlos, F.; Maycroft, K.; Stribbling, S. M.; Roussel, J.; Brueggen, J.; Stolz, B.; O'Reilly, T.; Wood, J.; Matter, A.; Marais, R.; Springer, C. J.

CORPORATE SOURCE: 1Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG

SOURCE: British Journal of Cancer (2003), 88(10), 1622-1630 CODEN: BJCAAI; ISSN: 0007-0920

PUBLISHER: Nature Publishing Group

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have generated fusion proteins between vascular endothelial growth factor (VEGF) and the **bacterial** enzyme carboxypeptidase G2 (CPG2) that can activate the prodrug 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid (CMDA). Three **asparagine** residues of CPG2 were mutated to glutamine (CPG2(Q)3) to **prevent glycosylation** during secretion, and truncations of VEGF165 were fused to either the C- or N-terminal of CPG2. The Km of the fusion proteins (37.5 .mu.M) was similar to that of secreted CPG2(Q)3 (29.5 .mu.M) but greater than that of wild-type CPG2 (8 .mu.M). The affinity of the fusion proteins for VEGF receptor-2 (VEGFR2) (Kd=0.5-1.1 nM) was similar to that of [125I]VEGF (Kd=0.5 nM) (ELISA) or slightly higher (Kd=1.3-9.6 nM) (competitive RIA). One protein, VEGF115-CPG2(Q)3-H6, possessed 140% of the enzymic activity of secreted CPG2(Q)3, and had a faster half-maximal binding time for VEGFR2 (77 s), than the other candidates (330 s). In vitro, VEGF115-CPG2(Q)3-H6 targeted CMDA cytotoxicity only towards VEGFR-expressing cells. The plasma half-life of VEGF115-CPG2(Q)3-H6 in vivo was 3 h, comparable to equiv. values obsd. in ADEPT. We conclude that enzyme prodrug therapy using VEGF as a targeting moiety represents a promising novel antitumor therapy, with VEGF115-CPG2(Q)3-H6 being a lead candidate.British Journal of Cancer (2003) 88, 1622-1630.

L31 ANSWER 4 OF 23 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 2003055556 MEDLINE

DOCUMENT NUMBER: 22452808 PubMed ID: 12565836

TITLE: Critical role of N-terminal N-glycosylation for proper folding of the human formyl peptide receptor.

AUTHOR: Wenzel-Seifert Katharina; Seifert Roland

CORPORATE SOURCE: Department of Pharmacology and Toxicology, The University

of Kansas, Lawrence, KS 66045-7582, USA.
CONTRACT NUMBER: 1 P20 RR15563 (NCRR)
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (2003 Feb 14) 301 (3) 693-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200304
ENTRY DATE: Entered STN: 20030205
Last Updated on STN: 20030416
Entered Medline: 20030414

AB The human formyl peptide receptor (FPR) is N-glycosylated and activates phagocytes via G(i)-proteins. The FPR expressed with G(i)alpha(2)beta(1)gamma(2) in Sf9 insect cells exhibits high constitutive activity as assessed by strong inhibitory effects of an inverse agonist and Na(+) on basal guanosine 5(')-O-(3-thiotriphosphate) (GTPgammaS) binding. The aim of our study was to analyze the role of N-glycosylation in FPR function. Site-directed mutagenesis of extracellular Asn residues **prevented** FPR **glycosylation** but not FPR expression in Sf9 membranes. However, in terms of high-affinity agonist binding, kinetics of GTPgammaS binding, number of G(i)-proteins activated, and constitutive activity, **non-glycosylated** FPR was much less active than native FPR. FPR-Asn4Gln/Asn10Gln/Asn179Gln and FPR-Asn4Gln/Asn10/Gln exhibited similar defects. Our data indicate that N-glycosylation of N-terminal Asn4 and Asn10 but not of Asn179 in the second extracellular loop is essential for proper folding and, hence, function of FPR. FPR deglycosylation by **bacterial** glycosidases could be a mechanism by which **bacteria** compromise host defense.

L31 ANSWER 5 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN DUPLICATE 5

ACCESSION NUMBER: 2003221601 EMBASE
TITLE: Campylobacter - A tale of two protein glycosylation systems.
AUTHOR: Szymanski C.M.; Logan S.M.; Linton D.; Wren B.W.
CORPORATE SOURCE: B.W. Wren, Dept. of Infect./Tropical Disease, London Sch. of Hyg./Trop. Medicine, Keppel St, London WC1 7HT, Canada. brendan.wren@lshtm.ac.uk
SOURCE: Trends in Microbiology, (1 May 2003) 11/5 (233-238).
Refs: 39
ISSN: 0966-842X CODEN: TRMIEA
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Post-translational **glycosylation** is a universal **modification** of proteins in eukarya, archaea and **bacteria**. Two recent publications describe the first confirmed report of a **bacterial** N-linked glycosylation pathway in the human gastrointestinal pathogen Campylobacter jejuni. In addition, an O-linked glycosylation pathway has been identified and characterized in C. jejuni and the related species Campylobacter coli. Both pathways have similarity to the respective N- and O-linked glycosylation processes in eukaryotes. In **bacteria**, homologues of the genes in both pathways are found in other organisms, the complex glycans linked to the glycoproteins share common biosynthetic precursors and these modifications could play similar

biological roles. Thus, Campylobacter provides a unique model system for the elucidation and exploitation of glycoprotein biosynthesis.

L31 ANSWER 6 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 6
ACCESSION NUMBER: 2002:896523 HCAPLUS
DOCUMENT NUMBER: 138:217981
TITLE: N-linked glycosylation in Campylobacter jejuni and its functional transfer into E. coli
AUTHOR(S): Wacker, Michael; Linton, Dennis; Hitchen, Paul G.; Nita-Lazar, Mihai; Haslam, Stuart M.; North, Simon J.; Panico, Maria; Morris, Howard R.; Dell, Anne; Wren, Brendan W.; Aebi, Markus
CORPORATE SOURCE: Department of Biology, Institute of Microbiology, Swiss Federal Institute of Technology, Zurich, CH-8092, Switz.
SOURCE: Science (Washington, DC, United States) (2002), 298(5599), 1790-1793
CODEN: SCIEAS; ISSN: 0036-8075
PUBLISHER: American Association for the Advancement of Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB N-linked protein **glycosylation** is the most abundant posttranslation **modification** of secretory proteins in eukaryotes. A wide range of functions are attributed to glycan structures covalently linked to **asparagine** residues within the **asparagine**-X-serine/threonine consensus sequence (Asn-Xaa-Ser/Thr). We found an N-linked glycosylation system in the **bacterium** Campylobacter jejuni and demonstrate that a functional N-linked glycosylation pathway could be transferred into Escherichia coli. Although the **bacterial** N-glycan differs structurally from its eukaryotic counterparts, the cloning of a universal N-linked glycosylation cassette in E. coli opens up the possibility of engineering permutations of recombinant glycan structures for research and industrial applications.
REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 7 OF 23 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2002124699 MEDLINE
DOCUMENT NUMBER: 21839645 PubMed ID: 11849543
TITLE: The Fap1 fimbrial adhesin is a glycoprotein: antibodies specific for the glycan moiety block the adhesion of Streptococcus parasanguis in an in vitro tooth model.
AUTHOR: Stephenson Aimee E; Wu Hui; Novak Jan; Tomana Milan; Mintz Keith; Fives-Taylor Paula
CORPORATE SOURCE: Department of Microbiology, University of Vermont, Burlington, VT, USA.
CONTRACT NUMBER: DK57750 (NIDDK)
R37-DE11000 (NIDCR)
SOURCE: MOLECULAR MICROBIOLOGY, (2002 Jan) 43 (1) 147-57.
Journal code: 8712028. ISSN: 0950-382X.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200205
ENTRY DATE: Entered STN: 20020226
Last Updated on STN: 20020511
Entered Medline: 20020510
AB Streptococcus parasanguis is a primary colonizer of the tooth surface and

plays a pivotal role in the formation of dental plaque. The fimbriae of S. parasanguis are important in mediating adhesion to saliva-coated hydroxylapatite (SHA), an in vitro tooth adhesion model. The Fap1 adhesin has been identified as the major fimbrial subunit, and recent studies suggest that Fap1 is a glycoprotein. Monosaccharide analysis of Fap1 purified from the culture supernatant of S. parasanguis indicated the presence of rhamnose, glucose, galactose, N-acetylglucosamine and N-acetylgalactosamine. A glycopeptide moiety was isolated from a pronase digest of Fap1 and purified by immunoaffinity chromatography. The monosaccharide composition of the purified glycopeptide was similar to that of the intact molecule. The functionality of the glycan moiety was determined using monoclonal antibodies (MAbs) specific for the intact Fap1 glycoprotein. These antibodies were grouped into two categories based on their ability to block adhesion of S. parasanguis to SHA and their corresponding specificity for either protein or glycan epitopes of the Fap1 protein. 'Non-blocking' MAb epitopes were mapped to unique protein sequences in the N-terminus of the Fap1 protein using **non-glycosylated** recombinant Fap1 proteins (rFap1 and drFap1) expressed in Escherichia coli. In contrast, the 'blocking' antibodies did not bind to the recombinant Fap1 proteins, and were effectively competed by the binding to the purified glycopeptide. These data suggest that the 'blocking' antibodies are specific for the glycan moiety and that the adhesion of S. parasanguis is mediated by sugar residues associated with Fap1.

L31 ANSWER 8 OF 23 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 2002495856 MEDLINE
DOCUMENT NUMBER: 22244717 PubMed ID: 12356469
TITLE: Functional homologs of cyanovirin-N amenable to mass production in **prokaryotic** and eukaryotic hosts.
AUTHOR: Mori Toshiyuki; Barrientos Laura G; Han Zhaozhong; Gronenborn Angela M; Turpin Jim A; Boyd Michael R
CORPORATE SOURCE: Molecular Targets Drug Discovery Program, NCI Center for Cancer Research, National Cancer Institute, NCI-Frederick, Frederick, MD 21702-1201, USA.. manuscripts@ncifcrf.gov
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (2002 Oct) 26 (1) 42-9.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200308
ENTRY DATE: Entered STN: 20021002
Last Updated on STN: 20030814
Entered Medline: 20030813

AB Cyanovirin-N (CV-N) is under development as a topical (vaginal or rectal) microbicide to prevent sexual transmission of human immunodeficiency virus (HIV), and an economically feasible means for very large-scale production of the protein is an urgent priority. We observed that **N-glycosylation** of CV-N in yeast **eliminated** the anti-HIV activity, and that dimeric forms and aggregates of CV-N occurred under certain conditions, potentially complicating the efficient, large-scale manufacture of pure monomeric CV-N. We therefore expressed and tested CV-N homologs in which the glycosylation-susceptible Asn residue at position 30 was replaced with Ala, Gln, or Val, and/or the Pro at position 51 was replaced by Gly to eliminate potential conformational heterogeneity. All homologs exhibited anti-HIV activity comparable to wild-type CV-N, and the Pro51Gly homologs were significantly more stable

proteins. These glycosylation-resistant, functional cyanovirins should be amenable to large-scale production either in **bacteria** or in eukaryotic hosts.

L31 ANSWER 9 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:833530 HCAPLUS
DOCUMENT NUMBER: 135:368550
TITLE: **Bacterial** carboxypeptidase G2 surface-tethered variants and their use in gene directed enzyme prodrug therapy
INVENTOR(S): Springer, Caroline Joy; Marais, Richard Malcolm; Spooner, Robert
PATENT ASSIGNEE(S): Cancer Research Ventures Limited, UK
SOURCE: PCT Int. Appl., 45 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001085960	A1	20011115	WO 2001-GB1988	20010504
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1280921	A1	20030205	EP 2001-925733	20010504
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: GB 2000-11060 A 20000508
WO 2001-GB1988 W 20010504

AB The present invention relates to **bacterial** carboxypeptidases for use in gene directed prodrug therapy (GDEPT), in particular for use in the treatment of disease, including tumors. Specifically, the invention relates to modified **bacterial** carboxypeptidases which have enhanced catalytic activity. Our GDEPT system (WO 96/40238) focuses on the use of the enzyme carboxypeptidase G2 (CPG2), from Pseudomonas strain RS16. CPG2 activates benzoic acid mustard prodrugs such as 4-[(2-chloroethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid (CMDA) to release L-glutamic acid and the DNA alkylating drug 4-[(2-chloroethyl)(2-mesoxyethyl)amino]benzoic acid, a potent cytotoxic agent. Tethering to the outer surface of the cells was achieved by fusing a mammalian secretion signal to the N-terminus of CPG2 and a receptor tyrosine kinase transmembrane domain to its C-terminus, to act as a membrane anchor. Thus CPG2 was transported through the Golgi/endoplasmic reticulum and inserted into the outer side of the plasma membrane and this form of CPG2 is referred to as surface-tethered CPG2 (stCPG2). However, stCPG2 was inappropriately glycosylated on three **asparagine** residues (N222, N264,N272) which resulted in redn. in enzymic activity. Some activity was restored by mutating these residues to glutamine to **prevent glycosylation** (referred to as stCPG2 (Q)3). The present invention relates to the further mutation of these **asparagine** residues which resulted in improved enzymic activity. Mutation of these residues

showed that the **asparagine** at position 264 (N264) was an important amino acid for maintaining dimer stability, whereas mutation of the **asparagines** at positions 222 and 272-(N222 and N272) has a less severe effect on dimer stability. The glutamine at position 264 in CPG2*(Q)3 was substituted with serine, threonine or alanine and dimer stability and enzyme activity were examd. Dimer stability was improved by the serine (CPG2*(QSQ)) substitution, whereas either the threonine (CPG2*(QTQ)) or alanine (CPG2*(QAQ)) did not restore dimer stability. CPG2*(QSQ) is almost twice as active as CPG2*(Q)3, but its apparent affinity for MTX was decreased by almost 6-fold (Table1). Furthermore, although CPG2*(QTQ) dimer stability was not improved, its catalytic activity was increased by ~2.5 fold, but it had a reduced apparent affinity for substrate (its Km was also increased by ~12 fold) compared to CPG2*(Q)3. We were intrigued to note that the surface tethering process appeared to overcome many of the detrimental effects induced by the N264 mutations.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L31 ANSWER 10 OF 23 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 2001160530 MEDLINE
DOCUMENT NUMBER: 21153200 PubMed ID: 11230125
TITLE: Arabidopsis glucosidase I mutants reveal a critical role of N-glycan trimming in seed development.
AUTHOR: Boisson M; Gomord V; Audran C; Berger N; Dubreucq B; Granier F; Lerouge P; Faye L; Caboche M; Lepiniec L
CORPORATE SOURCE: Laboratoire de Biologie des Semences, INRA-INAPG, Route de St-Cyr, 78026 Versailles, France.
SOURCE: EMBO JOURNAL, (2001 Mar 1) 20 (5) 1010-9.
Journal code: 8208664. ISSN: 0261-4189.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010425
Last Updated on STN: 20010425
Entered Medline: 20010419

AB Glycoproteins with **asparagine**-linked (N-linked) glycans occur in all eukaryotic cells. The function of their glycan moieties is one of the central problems in contemporary cell biology. **N-glycosylation** may **modify** physicochemical and biological protein properties such as conformation, degradation, intracellular sorting or secretion. We have isolated and characterized two allelic Arabidopsis mutants, gcs1-1 and gcs1-2, which produce abnormal shrunken seeds, blocked at the heart stage of development. The mutant seeds accumulate a low level of storage proteins, have no typical protein bodies, display abnormal cell enlargement and show occasional cell wall disruptions. The mutated gene has been cloned by T-DNA tagging. It codes for a protein homologous to animal and yeast alpha-glucosidase I, an enzyme that controls the first committed step for N-glycan trimming. Biochemical analyses have confirmed that trimming of the alpha1,2- linked glucosyl residue constitutive of the N-glycan precursor is blocked in this mutant. These results demonstrate the importance of N-glycan trimming for the accumulation of seed storage proteins, the formation of protein bodies, cell differentiation and embryo development.

L31 ANSWER 11 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:277839 BIOSIS

Search completed by David Schreiber 308-4292

Lucas 09/901,572

DOCUMENT NUMBER: PREV200100277839
TITLE: Asn to lys mutations at three sites which are N-glycosylated in the mammalian protein decrease the aggregation of Escherichia coli-derived erythropoietin.
AUTHOR(S): Narhi, Linda O. (1); Arakawa, Tsutomu; Aoki, Kenneth; Wen, Jie; Elliott, Steve; Boone, Thomas; Cheetham, Janet
CORPORATE SOURCE: (1) Amgen Inc., Thousand Oaks, CA, 91320: lnarhi@amgen.com USA
SOURCE: Protein Engineering, (February, 2001) Vol. 14, No. 2, pp. 135-140. print.
ISSN: 0269-2139.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Erythropoietin (EPO) derived from Escherichia coli is unstable to elevated temperature and tends to aggregate with time, making it unsuitable for high-resolution structure analysis. The mammalian EPO contains about 40% carbohydrate, which makes this protein more stable and less prone to aggregate than **non-glycosylated** E.coli-derived EPO, but makes it unsuitable for high-resolution analysis owing to its size and flexibility. In an attempt to decrease the aggregation of E.coli-derived EPO, the three **asparagine** residues at positions 24, 38 and 83 were mutated to lysine residues. In the native protein, these residues are the sites of N-linked glycosylation, which suggests that they should be located on the surface of the protein and should not be involved in interactions in the hydrophobic protein core. Therefore, the substitution of basic amino acids for these neutral **asparagine** residues is not expected to affect the protein structure, but should increase the isoelectric point of the protein and its net positive charge, decreasing its tendency to aggregate at or below neutral pH due to electrostatic interactions. No apparent alterations in receptor binding, as determined by both cell-surface receptor competition assay and in vitro receptor dimerization experiments, were observed when these mutations were introduced into the EPO sequence. However, this mutant protein displayed a significant increase in stability to heat treatment and to storage, relative to the wild-type molecule. This resulted in a greater number of observable cross peaks in the mutant EPO in 2D NOESY experiments. However, the mutant was similar to the wild-type in stability when urea was used as a denaturant. This indicates that the introduced mutations resulted in a decrease in aggregation with heating or with prolonged incubation at ambient temperature, without changing the conformational stability or the receptor binding affinity of the mutant protein. This approach of placing charged residues at sites where N-glycosylation occurs in vivo could be applied to other systems as well.

L31 ANSWER 12 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:231212 BIOSIS
DOCUMENT NUMBER: PREV200100231212
TITLE: Selective in vitro glycosylation of recombinant proteins: Semi-synthesis of novel homogeneous glycoforms of human erythropoietin.
AUTHOR(S): Macmillan, Derek; Bill, Roslyn M.; Sage, Karen A.; Fern, Dominic; Flitsch, Sabine L. (1)
CORPORATE SOURCE: (1) Department of Chemistry, University of Edinburgh, West Mains Road, Kings Buildings, Edinburgh, EH9 3JJ: s.flitsch@ed.ac.uk UK
SOURCE: Chemistry & Biology (London), (February, 2001) Vol. 8, No. 2, pp. 133-145. print.
ISSN: 1074-5521.

Search completed by David Schreiber 308-4292

Lucas 09/901,572

DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Background: A natural glycoprotein usually exists as a spectrum of glycosylated forms, where each protein molecule may be associated with an array of oligosaccharide structures. The overall range of glycoforms can have a variety of different biophysical and biochemical properties, although details of structure-function relationships are poorly understood, because of the microheterogeneity of biological samples. Hence, there is clearly a need for synthetic methods that give access to natural and unnatural homogeneously glycosylated proteins. The synthesis of novel glycoproteins through the selective reaction of glycosyl iodoacetamides with the thiol groups of cysteine residues, placed by site-directed mutagenesis at desired glycosylation sites has been developed. This provides a general method for the synthesis of homogeneously glycosylated proteins that carry saccharide side chains at natural or unnatural glycosylation sites. Here, we have shown that the approach can be applied to the glycoprotein hormone erythropoietin, an important therapeutic glycoprotein with three sites of N-glycosylation that are essential for in vivo biological activity. Results: Wild-type recombinant erythropoietin and three mutants in which glycosylation site **asparagine** residues had been changed to cysteines (His10-WThEPO, His10-Asn24Cys, His10-Asn38Cys, His10-Asn83CyshEPO) were overexpressed and purified in yields of 13 mg l-1 from Escherichia coli. Chemical glycosylation with glycosyl-beta-N-iodoacetamides could be monitored by electrospray MS. Both in the wild-type and in the mutant proteins, the potential side reaction of the other four cysteine residues (all involved in disulfide bonds) were not observed. Yield of glycosylation was generally about 50% and purification of glycosylated protein from **non-glycosylated** protein was readily carried out using lectin affinity chromatography. Dynamic light scattering analysis of the purified glycoproteins suggested that the glycoforms produced were monomeric and folded identically to the wild-type protein. Conclusions: Erythropoietin expressed in E. coli bearing specific Asn fwdarw Cys mutations at natural glycosylation sites can be glycosylated using beta-N-glycosyl iodoacetamides even in the presence of two disulfide bonds. The findings provide the basis for further elaboration of the glycan structures and development of this general methodology for the synthesis of semi-synthetic glycoproteins.

L31 ANSWER 13 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 2001077605 EMBASE
TITLE: In vivo glycosylation suppresses the aggregation of amyloidogenic hen egg white lysozymes expressed in yeast.
AUTHOR: Song Y.; Azakami H.; Hamasu M.; Kato A.
CORPORATE SOURCE: A. Kato, Dept. of Biological Chemistry, Yamaguchi University, Yamaguchi 753-8515, Japan. kato@agr.yamaguchi-u.ac.jp
SOURCE: FEBS Letters, (23 Feb 2001) 491/1-2 (63-66).
Refs: 11
ISSN: 0014-5793 CODEN: FEBLAL
PUBLISHER IDENT.: S 0014-5793(01)02151-2
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
013 Dermatology and Venereology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English

Search completed by David Schreiber 308-4292

SUMMARY LANGUAGE: English

AB The mutant hen egg white lysozymes Ile55Thr and Asp66His, corresponding to human amyloidogenic mutant lysozymes Ile56Thr and Asp67His, respectively, were secreted in Saccharomyces cerevisiae. The amyloidogenic mutants (I55T and D66H) of hen egg white lysozymes were remarkably less soluble than that of the wild-type protein. To enhance the secretion of these mutants, we constructed the glycosylated amyloidogenic lysozymes (I55T/G49N and D66H/G49N) having the N-glycosylation signal sequence (Asn-X-Ser) by the substitution of glycine with **asparagine** at position 49. The secretion of these glycosylated mutant proteins is greatly increased in S. cerevisiae, compared with that of **non-glycosylated** type. Both the glycosylated mutants retained about 40% enzymatic activity when incubated at pH 7.4 for 1 h at the physiological temperature of 37.degree.C whereas the **non-glycosylated** proteins eventually lost all activity under these conditions. These results suggest that the glycosylated chains could mask the .beta.-strand of amyloidogenic lysozymes from the intermolecular cross-.beta.-sheet association, thus improving the solubility of amyloidogenic lysozymes. .COPYRGT. 2001 Federation of European Biochemical Societies.

L31 ANSWER 14 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 10

ACCESSION NUMBER: 2000:376027 BIOSIS
DOCUMENT NUMBER: PREV200000376027
TITLE: Hydrogen bonding and catalysis: A novel explanation for how a single amino acid substitution can change the pH optimum of a glycosidase.
AUTHOR(S): Joshi, Manish D.; Sidhu, Gary; Pot, Isabelle; Brayer, Gary D.; Withers, Stephen G.; McIntosh, Lawrence P. (1)
CORPORATE SOURCE: (1) The Department of Biochemistry and Molecular Biology and the Protein Engineering Network of Centres of Excellence, University of British Columbia, Vancouver, BC, V6T 1Z3 Canada
SOURCE: Journal of Molecular Biology, (26 May, 2000) Vol. 299, No. 1, pp. 255-279. print.
ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The pH optima of family 11 xylanases are well correlated with the nature of the residue adjacent to the acid/base catalyst. In xylanases that function optimally under acidic conditions, this residue is aspartic acid, whereas it is **asparagine** in those that function under more alkaline conditions. Previous studies of wild-type (WT) Bacillus circulans xylanase (BCX), with an **asparagine** residue at position 35, demonstrated that its pH-dependent activity follows the ionization states of the nucleophile Glu78 (pKa 4.6) and the acid/base catalyst Glu172 (pKa 6.7). As predicted from sequence comparisons, substitution of this **asparagine** residue with an aspartic acid residue (N35D BCX) shifts its pH optimum from 5.7 to 4.6, with an apprx20% increase in activity. The bell-shaped pH-activity profile of this mutant enzyme follows apparent pKa values of 3.5 and 5.8. Based on 13C-NMR titrations, the predominant pKa values of its active-site carboxyl groups are 3.7 (Asp35), 5.7 (Glu78) and 8.4 (Glu172). Thus, in contrast to the WT enzyme, the pH-activity profile of N35D BCX appears to be set by Asp35 and Glu78. Mutational, kinetic, and structural studies of N35D BCX, both in its native and covalently **modified** 2-fluoro-xylobiosyl **glycosyl**-enzyme intermediate states, reveal that the xylanase still follows a double-displacement mechanism with Glu78 serving as the nucleophile. We

therefore propose that Asp35 and Glu172 function together as the general acid/base catalyst, and that N35D BCX exhibits a "reverse protonation" mechanism in which it is catalytically active when Asp35, with the lower pKa, is protonated, while Glu78, with the higher pKa, is deprotonated. This implies that the mutant enzyme must have an inherent catalytic efficiency at least 100-fold higher than that of the parental WT, because only apprx1% of its population is in the correct ionization state for catalysis at its pH optimum. The increased efficiency of N35D BCX, and by inference all "acidic" family 11 xylanases, is attributed to the formation of a short (2.7 ANG) hydrogen bond between Asp35 and Glu172, observed in the crystal structure of the glycosyl-enzyme intermediate of this enzyme, that will substantially stabilize the transition state for glycosyl transfer. Such a mechanism may be much more commonly employed than is generally realized, necessitating careful analysis of the pH-dependence of enzymatic catalysis.

L31 ANSWER 15 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 11
ACCESSION NUMBER: 1999:58301 BIOSIS
DOCUMENT NUMBER: PREV199900058301
TITLE: Exchange of Ser-4 for Val, Leu and Asn in the sequon Asn-Ala-Ser does not **prevent N-glycosylation** of the cell surface glycoprotein from Halobacterium halobium.
AUTHOR(S): Zeitler, Reinhard (1); Hochmuth, Eduard; Deutzmann, Rainer; Sumper, Manfred
CORPORATE SOURCE: (1) Inst. Anthropol. Humangenet., Univ. Frankfurt a.M., Siesmayerstr. 70, 60323 Frankfurt Germany
SOURCE: Glycobiology, (Dec., 1998) Vol. 8, No. 12, pp. 1157-1164. ISSN: 0959-6658.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The archaeon Halobacterium halobium expresses a cell surface glycoprotein (CSG) with a repeating pentasaccharide unit N-glycosidically linked via N-acetylgalactosamine to Asn-2 of the polypeptide (GalNAc(1-N)Asn linkage type). This **asparagine** of the linkage unit is located within the N-terminal sequence Ala-Asn-Ala-Ser-, in accordance with the tripeptide consensus sequence Asn-Xaa-Ser/Thr typical for nearly every N-glycosylation site known so far, which are of the GlcNAc(1-N)-Asn linkage type. By a gene replacement method csg mutants were created which replace the serine residue of the consensus sequence by valine, leucine, and **asparagine**. Unexpectedly, this elimination of the consensus sequence did not **prevent N-glycosylation**.
All respective mutant cell surface glycoproteins were N-glycosylated at Asn-2 with the same N-glycan chain as the wild type CSG. Asn-479 is N-glycosylated via a Glc(1-N)Asn linkage type in the wild type CSG. Replacement of Ser-481 in the sequence Asn-Ser-Ser for valine **prevented glycosylation** of Asn-479. From these results we postulate the existence of two different N-glycosyltransferases in H. halobium, one of which does not use the typical consensus sequence Asn-Xaa-Ser/Thr necessary for all other N-glycosyltransferases described so far.

L31 ANSWER 16 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 1998057555 EMBASE
TITLE: Consequences of the loss of O-linked glycosylation of meningococcal type IV pilin on piliation and pilus-mediated adhesion.
AUTHOR: Marceau M.; Forest K.; Beretti J.-L.; Tainer J.; Nassif X.

Search completed by David Schreiber 308-4292

CORPORATE SOURCE: X. Nassif, INSERM U411, Laboratoire Microbiologie, Fac. Medecine Necker-Enfants Malades, 156 Rue de Vaugirard, 75015 Paris, France. nassif@necker.fr
SOURCE: Molecular Microbiology, (1998) 27/4 (705-715).
Refs: 27
ISSN: 0950-382X CODEN: MOMIEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Pili, which are assembled from protein subunits called pilin, are indispensable for the adhesion of capsulated Neisseria meningitidis (MC) to eukaryotic cells. Both MC and Neisseria gonorrhoeae (GC) pilins are **glycosylated**, but the effect of this **modification** is unknown. In GC, a galactose .alpha.-1,3-N-acetyl glucosamine is O-linked to Ser-63, whereas in MC, an O-linked trisaccharide is present between residues 45 and 73 of pilin. As Ser-63 was found to be conserved in pilin variants from different strains, it was replaced by Ala in two MC variants to test the possible role of this residue in pilin glycosylation and modulation of pili function. The mutated alleles were stably expressed in MC, and the proteins they encoded migrated more quickly than the normal protein during SDS-PAGE. As controls, neighbouring Asn-61 and Ser-62 were replaced by an Ala with no effect on electrophoretic mobility. Silver staining of purified pilin obtained from MC after oxidation with periodic acid confirmed the loss of glycosylation in the Ser-63.fwdarw.Ala pilin variants. Mass spectrometry of HPLC-purified trypsin-digested peptides of pilin and Ser-63.fwdarw.Ala pilin confirmed that peptide 45-73 has the molecular size of a glycopeptide In the wild type. In strains producing **non-glycosylated** pilin variants, we observed that (i) no truncated S pilin monomer was produced; (ii) piliation was slightly increased; and (iii) presumably as a consequence, adhesiveness for epithelial cells was increased 1.6- to twofold in these derivatives. In addition, pilin monomers and/or individual pilus fibres, obtained after solubilization of a crude pill preparation in a high pH buffer, were reassociated into insoluble aggregates of pili more completely with **non-glycosylated** variants than with the normal pilin. Taken together, these data **eliminate** a major role for pilin **glycosylation** in piliation and subsequent pilus-mediated adhesion, but they demonstrate that glycosylation facilitates solubilization of pilin monomers and/or individual pilus fibres.

L31 ANSWER 17 OF 23 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 1999087325 MEDLINE
DOCUMENT NUMBER: 99087325 PubMed ID: 9872320
TITLE: An asparaginyl endopeptidase processes a microbial antigen for class II MHC presentation.
COMMENT: Comment in: Nature. 1998 Dec 17;396(6712):625, 627
AUTHOR: Manoury B; Hewitt E W; Morrice N; Dando P M; Barrett A J; Watts C
CORPORATE SOURCE: Department of Biochemistry, University of Dundee, UK.
SOURCE: NATURE, (1998 Dec 17) 396 (6712) 695-9.
Journal code: 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990202

Search completed by David Schreiber 308-4292

Last Updated on STN: 20000303
Entered Medline: 19990119

AB Foreign protein antigens must be broken down within endosomes or lysosomes to generate suitable peptides that will form complexes with class II major histocompatibility complex molecules for presentation to T cells. However, it is not known which proteases are required for antigen processing. To investigate this, we exposed a domain of the microbial tetanus toxin antigen (TTCF) to disrupted lysosomes that had been purified from a human B-cell line. Here we show that the dominant processing activity is not one of the known lysosomal cathepsins, which are generally believed to be the principal enzymes involved in antigen processing, but is instead an **asparagine**-specific cysteine endopeptidase. This enzyme seems similar or identical to a mammalian homologue of the legumain/haemoglobinase asparaginyl endopeptidases found originally in plants and parasites. We designed competitive peptide inhibitors of B-cell asparaginyl endopeptidase (AEP) that specifically block its proteolytic activity and inhibit processing of TTCF in vitro. In vivo, these inhibitors slow TTCF presentation to T cells, whereas preprocessing of TTCF with AEP accelerates its presentation, indicating that this enzyme performs a key step in TTCF processing. We also show that N-glycosylation of **asparagine** residues blocks AEP action in vitro. This indicates that **N-glycosylation** could **eliminate** sites of processing by AEP in mammalian proteins, allowing preferential processing of microbial antigens.

L31 ANSWER 18 OF 23 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:532899 BIOSIS
DOCUMENT NUMBER: PREV199598547199
TITLE: Overexpression and purification of **non-glycosylated** recombinant endo-beta-N-acetylglucosaminidase F-3.
AUTHOR(S): Tarentino, A. I.; Quinones, G.; Plummer, T. H., Jr.
CORPORATE SOURCE: Div. Mol. Med., Wadsworth Cent. Lab. Res., N.Y. State Dep. Health, Albany, NY 12201-0509 USA
SOURCE: Glycobiology, (1995) Vol. 5, No. 6, pp. 599-601. ISSN: 0959-6658.
DOCUMENT TYPE: Article
LANGUAGE: English

AB The gene for endo-beta-N-acetylglucosaminidase F-3 was cloned into the high-expression vector pMAL c-2, and expressed in Escherichia coli as a fusion protein. A key step in the purification employed Poros II (HS) chromatography, which greatly facilitated isolation of the enzyme from crude intracellular lysates. The unfused enzyme was recovered following digestion with Factor X-a and was isolated in a homogeneous form. The enzyme is **non-glycosylated** and fully active, and is a very useful analytical tool for investigating the structure of **asparagine**-linked glycans, especially those with coresubstituted alpha-1,6 fucosyl residues.

L31 ANSWER 19 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 13
ACCESSION NUMBER: 1995:802948 HCAPLUS
DOCUMENT NUMBER: 123:218626
TITLE: **Bacterial** expression of human chorionic gonadotropin .alpha. subunit: studies on refolding, dimer assembly and interaction with two different .beta. subunits
AUTHOR(S): Ren, Peifeng; Sairam, M. R.; Yarney, T. A.
CORPORATE SOURCE: Reproduction Research Laboratory, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal,

Quebec, Can.
SOURCE: Molecular and Cellular Endocrinology (1995), 113(1), 39-51
CODEN: MCEND6; ISSN: 0303-7207
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human chorionic gonadotropin (hCG) is a member of a family of heterodimeric glycoprotein hormones that have a common .alpha. subunit but differ in their hormone-specific .beta. subunit. The common .alpha. subunit contains two **asparagine** (N)-linked oligosaccharides. To study the function of carbohydrates on in vitro refolding of .alpha. subunit and dimer assembly, the authors generated recombinant **non -glycosylated** hCG .alpha. subunit (rNG-hCG.alpha.) from E. coli. The expression vector was constructed by inserting hCG.alpha. cDNA coding for the mature form in-frame into a pQE-30 vector, which contains a 6 .times. His sequence immediately before the 5'-end of hCG.alpha. cDNA for subsequent purifn. of rNG-hCG.alpha.. The rNG-hCG.alpha. expressed in inclusion bodies was efficiently purified by immobilized metal chelate affinity chromatog. on Ni-NTA resin. SDS-PAGE, solid-phase binding assay and immunoblotting demonstrated the expression of rNG-hCG. Its .alpha. mol. wt. on SDS-PAGE was 14.7 kDa under reducing conditions and 15 kDa for a monomer accompanied with some higher mol. wt. oligomer under non-reducing conditions. Reconstitution of rNG-hCG.alpha. with native hCG.beta. and oFSH.beta. occurred in very low yield under std. conditions. However, the oxidn.-redn. system cystamine (1.34 mM) and cysteamine (7.3 mM) facilitated both the refolding of rNG-hCG.alpha. and reconstitution of rNG-hCG.alpha. with native hCG.beta. to regain partially correct conformation. These were revealed by conformationally sensitive antibody and receptor binding assays. Cystamine and cysteamine were more effective in the recombination of rNG-hCG.alpha. with oFSH.beta. as indicated by a 22-36-fold decrease in the amt. required to cause a 50% competitive inhibition in radioreceptor assay. They have no effect on assembly of rNG-hCG.alpha. with oLH.beta.. The results suggest the carbohydrate moieties confer greater conformational flexibility to the backbone of the .beta. subunit and the relative rigidity of the .beta. subunit may serve as a conformational template of the .alpha. subunit. The present approach has made it possible to prep. the **non-glycosylated** gonadotropin .alpha. subunit in adequate amts. for further study on their biol. and topog. features in complete absence of carbohydrate.

L31 ANSWER 20 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 14

ACCESSION NUMBER: 1994:186087 HCAPLUS
DOCUMENT NUMBER: 120:186087
TITLE: Characterization of common carbohydrate antigenic determinants on soya bean cell-wall enzymes
AUTHOR(S): Teissere, Marcel; Nari, Joannes; Ferte, Nathalie; Mutaftschiev, Stephanie; Noat, Georges
CORPORATE SOURCE: Cent. Biochim. Bio. Mol., Cent. Natl. Rech. Sci., Marseille, F-13402, Fr.
SOURCE: Plant and Cell Physiology (1994), 35(1), 121-5
CODEN: PCPHA5; ISSN: 0032-0781
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Three soya-bean (Glycine max) cell-wall enzymes (.beta.-glucosidase, pectin Me esterase and phosphatase) have been found to be glycoproteins. The polyclonal antibodies raised against pectin Me esterase and .beta.-glucosidase lacked specificity, cross-reacted highly with native enzymes and also both reacted with pure soya-bean phosphatase, horseradish

peroxidase and honeybee venom phospholipase A2. They did not react with either **non-glycosylated bacterial** phosphatase or deglycosylated cell-wall enzymes. The two antisera contained both non-specific anti-glycan antibodies and specific anti-polypeptide antibodies that were quantified. Antiglycan antibodies specific to .alpha.1-3 fucose and .beta.1-2 xylose were detected in both antisera and were sepd. and quantified. The occurrence of terminal fucose (and mannose) was confirmed with specific lectins. These results indicate that most of the common glycan epitopes probably correspond to the **asparagine**-linked complex glycan previously detected in several glycoproteins of plants as well as in those of molluscs and insects.

L31 ANSWER 21 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
DUPLICATE 15
ACCESSION NUMBER: 92122167 EMBASE
DOCUMENT NUMBER: 1992122167
TITLE: S-layer of Lactobacillus helveticus ATCC 12046: Isolation, chemical characterization and re-formation after extraction with lithium chloride.
AUTHOR: Lortal S.; Van Heijenoort J.; Gruber K.; Sleytr U.B.
CORPORATE SOURCE: INRA, Laboratoire de Recherches, de Technologie Laitiere, 65 rue de St-Brieuc,35042 Rennes cedex, France
SOURCE: Journal of General Microbiology, (1992) 138/3 (611-618). ISSN: 0022-1287 CODEN: JGMIAN
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In a previous study, electron microscopic examinations of thin sections of Lactobacillus helveticus ATCC 12046 revealed a three-layered structure of the cell wall. The outermost component was identified as a layer of a **non-glycosylated** 52 kDa protein. Freeze-etched preparations of intact cells have now demonstrated that this protein layer is an oblique surface layer (S-layer) lattice (a = 4.5nm, b = 9.6nm, y = 77.degree.) which completely covers the cell surface. Treatment with 5 M-LiCl extracted the S-layer protein from intact cells efficiently and selectively. Viability did not decrease significantly. Moreover, the S-layer reappeared when treated cells were allowed to grow again. In vitro self-assembly products obtained upon aggregation of isolated S-layer subunits exhibited the same oblique S-layer symmetry as observed on intact cells in vivo. The purified S-layer protein had a high content (44%) of hydrophobic amino acids. The N-terminal sequence was mainly composed of alanine, threonine, **asparagine** and aspartic acid.

L31 ANSWER 22 OF 23 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V. on STN
ACCESSION NUMBER: 91268265 EMBASE
DOCUMENT NUMBER: 1991268265
TITLE: Studies on the biotin-binding site of avidin Minimized fragments that bind biotin.
AUTHOR: Hiller Y.; Bayer E.A.; Wilchek M.
CORPORATE SOURCE: Department of Biophysics, Weizmann Institute of Science,Rehovot 76100, Israel
SOURCE: Biochemical Journal, (1991) 278/2 (573-585). ISSN: 0264-6021 CODEN: BIJOAK
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LANGUAGE: English
SUMMARY LANGUAGE: English

AB The object of this study was to define minimized biotin-binding fragments, or 'prorecognition sites', of either the egg-white glycoprotein avidin or its **bacterial** analogue streptavidin. Because of the extreme stability to enzymic hydrolysis, fragments of avidin were prepared by chemical means and examined for their individual biotin-binding capacity. Treatment of avidin with hydroxylamine was shown to result in new cleavage sites in addition to the known Asn-Gly cleavage site (position 88-89 in avidin). Notably, the Asn-Glu and Asp-Lys peptide bonds (positions 42-43 and 57-58 respectively) were readily cleaved; in addition, lesser levels of hydrolysis of the Gln-Pro (61-62) and Asn-Asp (12-13 and 104-105) bonds could be detected. The smallest biotin-binding peptide fragment, derived from hydroxylamine cleavage of either native or **non-glycosylated** avidin, was identified to comprise residues 1-42. CNBr cleavage resulted in a 78-amino acid-residue fragment (residues 19-96) that still retained activity. The data ascribe an important biotin-binding function to the overlapping region (residues 19-42) of avidin, which bears the single tyrosine moiety. This contention was corroborated by synthesizing a tridecapeptide corresponding to residues 26-38 of avidin; this peptide was shown to recognize biotin. Streptavidin was not susceptible to either enzymic or chemical cleavage methods used in this work. The approach taken in this study enabled the experimental distinction between the chemical and structural elements of the binding site. The capacity to assign biotin-binding activity to the tyrosine-containing domain of avidin underscores its primary chemical contribution to the binding of biotin by avidin.

L31 ANSWER 23 OF 23 HCAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1988:199486 HCAPLUS
DOCUMENT NUMBER: 108:199486
TITLE: Cloning and expression of mutant tissue-type plasminogen activator cDNA in insect and mammalian cells
INVENTOR(S): Larsen, Glenn R.; Ahern, Tim J.
PATENT ASSIGNEE(S): Genetics Institute, Inc., USA
SOURCE: PCT Int. Appl., 80 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
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WO 8704722	A1	19870813	WO 1987-US257	19870130
W: AU, DK, FI, JP, NO, US, US, US, US				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
US 5002887	A	19910326	US 1986-882051	19860703
AU 8772395	A1	19870825	AU 1987-72395	19870130
AU 612974	B2	19910725		
JP 63501335	T2	19880526	JP 1987-502352	19870130
JP 2527454	B2	19960821		
EP 293394	A1	19881207	EP 1987-902884	19870130
EP 293394	B1	19940420		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
AT 104700	E	19940515	AT 1987-902884	19870130
JP 10004960	A2	19980113	JP 1997-73177	19870130
ES 2004438	A6	19890101	ES 1987-1920	19870701

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DK 8705118	A	19870929	DK 1987-5118	19870929
NO 8704091	A	19870929	NO 1987-4091	19870929
NO 175317	B	19940620		
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US 5071972	A	19911210	US 1988-382678	19881019
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JP 05268959	A2	19931019	JP 1992-149677	19920609
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JP 07046989	A2	19950221	JP 1994-88848	19940426
JP 2568382	B2	19970108		

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	US 1986-853781	19860418
	US 1986-861699	19860509
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	EP 1987-902884	19870130
	JP 1992-149677	19870130
	WO 1987-US257	19870130
	US 1988-382678	19881019
	US 1991-805415	19911210

AB Novel thromobolytic proteins with tissue-type plasminogen activator (tPA) activity are expressed from mutagenized cDNA in insect or mammalian cells. The tPA gene from mammalian expression vector pSVPA4 was excised with SacI, and inserted into M13mp8, which was used to transform E. coli JM101. Single-stranded DNA isolated from the cells were used for site-specific mutagenesis to change **asparagine**-117 to glutamine-117 and to delete residues Cys 6-Ser 50 inclusive. The mutant tPA cDNA was then inserted into the insect cell expression vectors pIVPA/1 to prep. pIVPA/.DELTA.FBR;Gln117. Recombinant nuclear polyhedrosis virus contg. the mutant gene was prepd. by cotransfection of Spodoptera cells with the plasmid and virus. The mutant tPA was expressed in virus-infected Spodoptera cells.